Soil carbon loss by experimental warming in a tropical forest

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Soil carbon loss from a tropical forest in response to *in situ* experimental warming

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Tropical soils contain a third of global soil carbon[1], so destabilization of soil organic matter caused by the approximate 4°C warming predicted for tropical regions this century could accelerate climate change by releasing additional carbon dioxide (CO_2) to the atmosphere[2-5]. Theory predicts that warming should cause only modest carbon loss in tropical soils relative to those at higher latitudes[4,6], but there have been no warming experiments in tropical forests to test this prediction[7]. Here we show that *in situ* experimental warming of a lowland tropical forest soil on Barro Colorado Island, Panama, caused an unexpectedly large increase in soil CO_2 emissions. Two years of warming of the whole soil profile by 4°C increased CO_2 emission by 55% compared to soils at ambient temperature. The additional CO_2 originated from heterotrophic rather than autotrophic sources and equated to a loss of 8.2 ± 4.2 (± 1 SE) Mg C ha^{-1} yr^{-1} from the breakdown of soil organic matter. During this time, we detected no acclimation of respiration rates, no thermal compensation or change in temperature sensitivity of enzyme activities, and no change in microbial carbon-use efficiency. These results demonstrate a high sensitivity of soil carbon in tropical forests to warming, which represents a potentially substantial positive feedback to climate change.

Tropical forests play a large role in the global carbon (C) cycle, because they exchange more CO_2 with the atmosphere than any other ecosystem, contain over two-thirds of terrestrial plant biomass[8] and harbour over a quarter of global soil C (ref. 1). Between 30 and 50% of the C respired from tropical forests originates from soil, most of which is derived from the decomposition of organic matter[9-11]. Thus, even a small increase in respiration from tropical forest soils could have a large effect on atmospheric CO_2 concentrations, with consequences for global climate.

There is considerable concern that increased global temperatures will destabilize soil C and increase the flux of CO_2 from soil to the atmosphere[2-5]. Experiments in temperate and arctic regions have consistently found that short-term (< 2 years) warming increases the soil CO_2 efflux by an average of 46 ± 8% compared to soil at ambient temperature[2,3,5,12]. For the tropics, it is expected that the response of soil C to warming will be smaller than at higher latitudes, because kinetic theory predicts that the intrinsic temperature sensitivity of reaction rates is reduced at higher temperatures[4,13], and meta-analyses of warming experiments have shown that the temperature sensitivity of soil C loss increases with latitude[6]. However, the extent to which intrinsic temperature sensitivity translates into actual ('apparent') temperature sensitivity depends on co-variation of other environmental factors that influence respiration, such as soil moisture and substrate availability[4,14]. As there have been no *in situ* warming experiments conducted in tropical forests, the apparent temperature sensitivity of soil organic matter in this biome remains unknown. As a result, earth-system models continue to use kinetic theory to define the temperature sensitivity of soil C[15], limiting how they predict the response of tropical forests to global environmental change[16,17].

Several factors could influence the apparent temperature sensitivity of soil organic matter. For example, soil warming is typically accompanied by soil drying, which can either reduce respiration in aerobic soils by reducing water availability, or increase respiration in waterlogged soils by increasing oxygen availability[4,7,14]. Warming can also affect respiration rates by inducing changes in biotic processes, such as the physiological response or community composition of microbes, or changes in

53 substrate availability to decomposers[13]. In experiments performed at higher latitudes, temperature-
54 adaptive or compensatory responses of microbial communities and enzyme activities have been shown
55 to modulate the effect of warming on the soil C cycle[2,13]. In the tropics, future novel warm temperature
56 maxima could exceed critical biochemical thresholds[7,18], with added complexity emerging from altered
57 interactions among species-rich plant and microbial communities[19], and from covarying changes in
58 hydrological and nutrient cycles[7].

59 Here, we present results from the first soil warming experiment in a lowland tropical forest
60 (SWELTR: Soil Warming Experiment in Lowland Tropical Rainforest). The experiment tests the
61 response of the whole soil profile to the 4ºC warming predicted for tropical latitudes by the end of this
62 century[16] (Fig. 1A; Extended Data Figs. 1-3). SWELTR consists of five pairs of circular control and
63 warmed plots, evenly distributed within approximately 1 ha of seasonally-moist lowland tropical forest
64 on Barro Colorado Island, Panama. The soils are moderately weathered Dystric Eutrudepts
65 (Inceptisols) that have developed on the volcanic facies of the Bohio Formation (Extended Data Table
66 1, see methods). Each warmed plot has a ground surface area of ~20 m^2 heated to 1.2 m depth, resulting
67 in a total of 120 m^3 of warmed soil across the experiment.

68 Two years of experimental warming increased soil CO_2 emissions by 55%, from 18.8 ± 1.9 Mg
69 C ha^{-1} yr^{-1} in control plots to 29.2 ± 5.0 Mg C ha^{-1} yr^{-1} in warmed plots (treatment effect, $p < 0.05$; Fig.
70 2; Extended Data Table 2). The soil CO_2 emission rate from unheated (i.e., control) plots is
71 representative of tropical forests worldwide (8–40 Mg C ha^{-1} yr^{-1}), including in the Amazon basin (12–
72 24 Mg C ha^{-1} yr^{-1})[20]. Using exclusion and ingrowth cores to partition respiration from heterotrophic
73 (soil-derived) and autotrophic (root-derived) sources, we find that the increase in CO_2 efflux was
74 derived predominantly from heterotrophic sources, whether from the decomposition of fresh-litter
75 inputs or pre-existing soil organic matter (Figs. 2-3, Extended Data Fig. 5). Soil-derived respiration
76 increased from 12.0 ± 2.1 Mg C ha^{-1} yr^{-1} in control plots to 20.1 ± 4.2 Mg C ha^{-1} yr^{-1} in warmed plots
77 (a 68% increase of 8.2 Mg C ha^{-1} yr^{-1}; treatment effect, $p < 0.05$), while root-derived respiration was

not altered significantly (p = 0.21; 6.8 ± 1.2 and 9.0 ± 3.4 Mg C ha^{-1} yr^{-1} in control and warmed plots, respectively; Fig. 3, Extended Data Table 3).

It is possible that the large warming-induced increase in soil CO_2 efflux was due in part to soil drying, because the warmed plots were slightly drier than the controls, particularly in the early wet season (Extended Data Fig. 3). In wet soils, soil drying can increase respiration by increasing the supply of oxygen to heterotrophic microbes. In contrast, soil drying under aerobic conditions can reduce heterotrophic respiration by promoting water limitation[20]. Here, we find a marginally non-significant effect of soil moisture on CO_2 efflux across seasons (Extended Data Table 2; annual p = 0.69, wet-season p = 0.07 and dry-season p = 0.06), consistent with the parabolic relationship of CO_2 flux with soil moisture for this site[20]. However, there was no direct effect of warming on soil moisture (for surface soils p = 0.19, whole-profile p = 0.24; Extended Data Tables 4-5), and the interaction between soil moisture and warming in the CO_2 efflux model was not significant (annually and for individual seasons; p > 0.2; Extended Data Table 2), indicating that the warming effect on CO_2 efflux was not influenced by soil moisture. Furthermore, soil moisture was not correlated with soil CO_2 efflux in the warmed plots (Extended Data Fig. 3); and drying during the early wet season in warmed soil (Extended Data Fig. 3) should decrease rather than increase CO_2 efflux, because the soil was aerobic during this period and below the moisture content of 0.45 m^3 m^{-3} at which soil CO_2 efflux peaks in this forest[20]. Our data thus show that although soil moisture influenced soil CO_2 efflux and that warmed plots were slightly drier than control plots, particularly during the early wet season, this did not contribute significantly to the increased CO_2 efflux from warmed soil.

There was no moderation of the warming-induced increase in soil CO_2 efflux over the two years of the experiment. Such a moderation might be expected in the long-term, whether through substrate limitation, adaptation of microbial communities (through changes in microbial carbon-use-efficiency; CUE) or thermal compensation of enzyme activities (reduced V_{max} at higher temperatures)[2,13,21]. We found no reduction in extractable or mineralized nitrogen or phosphorus with

warming, as would be expected under nutrient limitation (Extended Data Figs. 6-7). Almost all hydrolytic enzymes were unaffected by warming (Extended Data Figs. 6-7), except for β-xylanase – an enzyme involved in hemicellulose degradation – for which activity increased with warming during the wet season, an opposite response to that predicted by thermal compensation[19]. The temperature sensitivity of enzyme activity (Q_{10} of V_{max}) was unaffected by warming (Extended Data Fig. 8), indicating no dampening effect on soil C breakdown as a result of decreased enzyme flexibility (which would cause decreased affinity of the enzyme for its substrate), as expected at warmer temperatures[22]. Microbial CUE, which broadly represents C stabilised in biomass relative to C lost in respiration and can promote long-term (decadal) soil C loss[2,21], was unaffected by warming (Treatment effect, p = 0.37; Extended Data Figs. 6-7). Indeed, microbial C increased with warming at the annual scale (treatment effect, annual scale, p = 0.02; with a marginally non-significant increase at the seasonal scale, $p < 0.1$ Extended Data Fig. 7), suggesting slightly increased growth in response to greater organic matter turnover in the absence of nutrient constraints to C degradation, which did not translate into changes in CUE.

Our finding that tropical forest soil C has a high apparent temperature sensitivity challenges the prevailing expectation that the temperature sensitivity of soil C is lower in the tropics compared to cooler ecosystems at higher latitudes[4,6]. The 55% increase in total soil CO_2 emissions we report here following two years of 4ºC whole-profile warming (18.8 to 29.2 Mg C ha^{-1} yr^{-1}) is larger than that found in a temperate forest using a similar whole-soil-profile experimental design (34–37% increase over two years of 4ºC whole-profile warming; 13 to 17.5 Mg C ha^{-1} yr^{-1})[5]. In addition, the rate of additional soil C loss (8.2 Mg C ha^{-1} yr^{-1}) is greater than for all the studies in a recent meta-analysis of surface-only soil warming experiments at higher latitude sites (all loss rates < 5 Mg C ha^{-1} yr^{-1})[3]. The expectation that the temperature sensitivity of soil C breakdown is lower in the tropics compared to higher latitudes, based in part on kinetic theory and commonly used to describe soil C responses in earth-system models[15], is therefore not consistent with the ('apparent')[4,14] temperature sensitivity of

the breakdown of tropical forest soil C reported here. Our results are, however, consistent with recent atmospheric and satellite measurements, which have shown a high sensitivity of ecosystem-scale C cycling in tropical regions in response to interannual temperature variation[23,24]. Our findings suggest that soils contribute a major component of these ecosystem-scale responses to warming.

This high apparent temperature sensitivity of tropical forest soil C under *in situ* experimental warming must arise through the temperature response of covarying ecosystem properties rather than as the sole consequence of intrinsic kinetic processes. Although our data do not provide conclusive mechanistic evidence for the marked increase in soil C loss from warmed soil, several findings point to a possible explanation: i) the general absence of thermal compensation in enzyme activities (no decrease in V_{max}); ii) no change in the temperature sensitivity of enzymes under warming (no decrease in Q_{10} of V_{max}); and iii) no moderating thermal response of microbial CUE (Extended Data Figs. 6-7)[21,25,26]. Together, our results indicate that organic matter degradation increased under warming with no moderating responses or acclimation during our study period among microbial communities, or the enzymes they synthesise.

This surprisingly large loss of soil C from warmed soil represents a substantial positive climate feedback over the period of this study. The additional C loss from warming observed here is of similar magnitude to annual litterfall-C inputs at this site (5–7 Mg C ha^{-1} yr^{-1})[27] and is equivalent to approximately 13% of the total soil C stock, or 30% of gross primary productivity (27.5 Mg C ha^{-1} yr^{-1})[28]. Extrapolation of the first two years of C loss in our experiment across the entire tropical forest soil C stock (502 Pg C)[1] indicates a global loss of >65 Pg C with 4ºC warming this century, which is consistent with estimated C loss based on a five-year soil translocation experiment in tropical forests elsewhere[29]. In the light of these findings, earlier estimates of global soil C loss under 4ºC warming, which were based on experiments performed at higher latitudes (120–190 Pg C)[2,30], underestimate the magnitude of this global earth-atmosphere feedback.

We expect that the rate of soil C loss will eventually decline in warmed soils as substrate limitation increases, but we do not know how long this will take, nor whether the long-term soil C balance will be affected by plant-soil interactions or changes in soil microbial communities as they adapt to warmer temperatures[2,12,13,21]. The nature of these longer-term responses will determine the strength of this positive earth-atmosphere feedback, already significant in the short-term, in contributing to further climate warming.

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Supplementary Information is linked to the online version of the paper

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Author contributions | ATN conceived the study with BLT and PM. ATN, EV and BLT performed the study. ATN analysed the data and wrote the paper with BLT and PM.

Author Information | The authors declare no competing financial interests. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to A.T.N. (anotting@ed.ac.uk).

Data availability statement | The data supporting the findings of this study are available within the paper and its supplementary information files, with further information available from the corresponding author upon reasonable request.

Supplementary Information and Extended Data for this manuscript include the following:

- **Supplementary Materials and Methods**
- **Extended Data Table 1 to 5**
- **Extended Data Figs. 1 to 8**

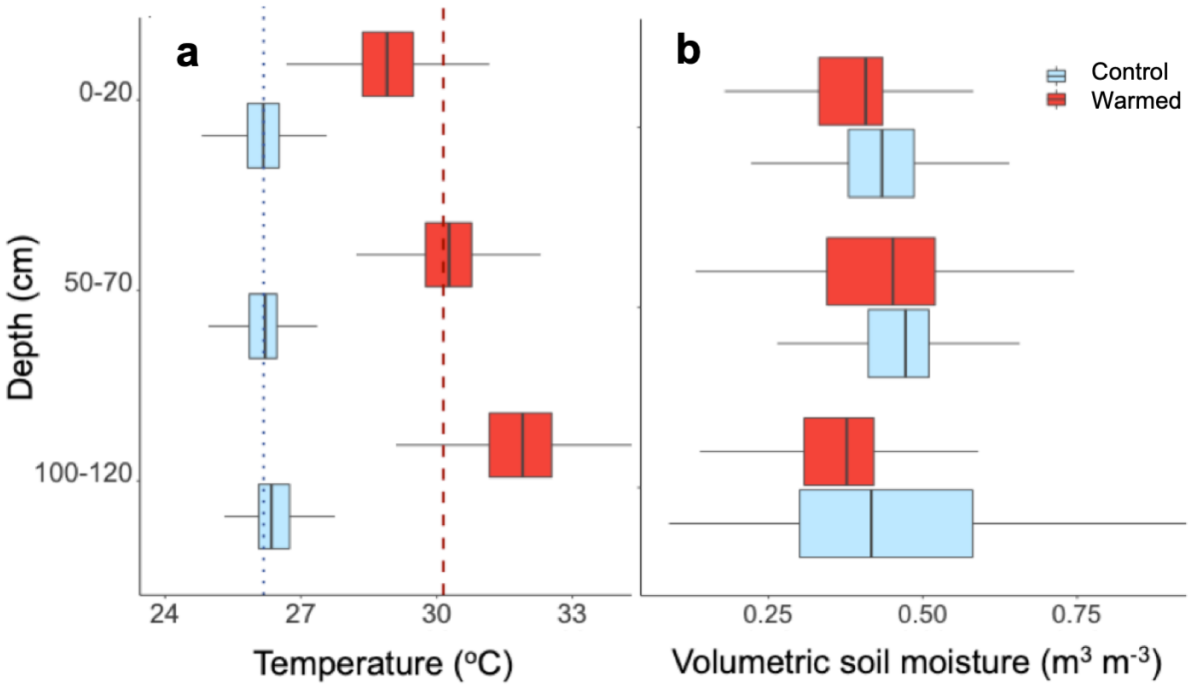

Figure 1 | Mean differences in (a) soil temperature and (b) soil moisture content between control and warmed plots by depth. Data are the means of continuous measurements from integrated soil temperature and moisture probes (Campbell CS655) for the two-year period after the warming treatment began (Dec 2016 – Dec 2018). The error bars represent one standard error of the temporal variation across five plots (Treatment effect $p < 0.001$ for all comparisons, based on the temporal variation across n = 5 plots). The vertical lines show the mean soil temperature across the soil profile, which is 26.18°C for control plots and 30.14°C for warmed plots (3.97°C difference).

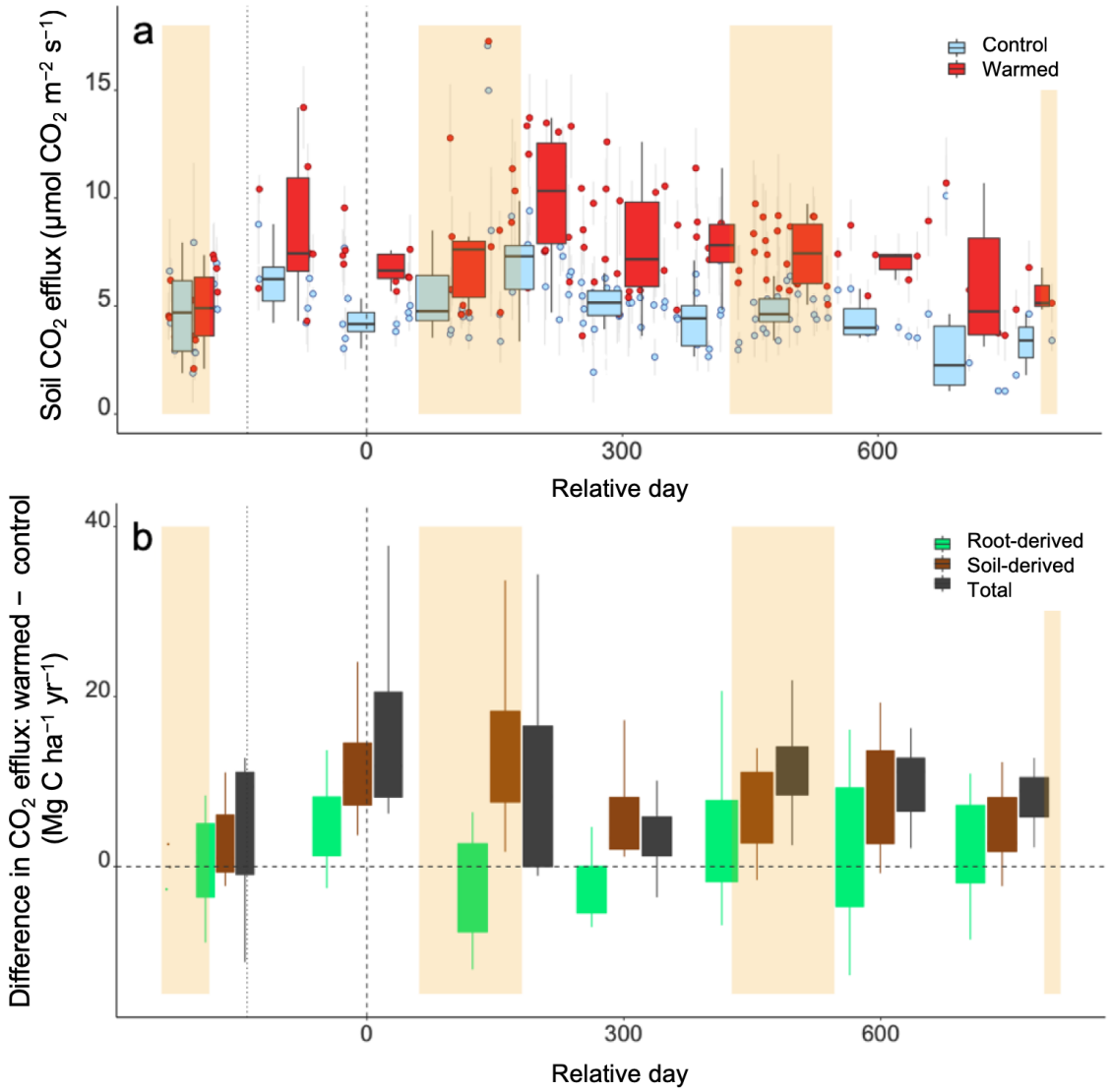

Figure 2 | Soil CO_2 efflux from control and warmed soils over two years. Panel (a) shows the total soil CO_2 flux during the study period (2017-2019). Panel (b) shows the total soil CO_2 flux partitioned into soil-derived and root-derived components (b), relative to the beginning of the warming treatment. Measurements were made every one or two weeks. Points represent the mean value of five plots, with error bars representing one standard error of the spatial variation. The box plots represent the mean and temporal variation over sequential 100-day periods to show seasonal dynamics. The dotted vertical line (relative day = –150) is when installation and testing of warming plots began (during this period each plot was warmed by 4°C relative to controls for a period of 1-2 weeks); the dashed vertical line (relative day = 0; 1 November 2017) shows when all five warming plots were switched on permanently. The shaded areas represent dry seasons (1 January–1 April). Soil CO_2 efflux was significantly higher in warmed plots for annual data and for dry or wet seasons individually (Extended Data Table 2). The error bars for points represent one standard error of the spatial variation (n = 5 plots); the error bars for boxes represent one standard error of the temporal variation (averaged over 100 days).

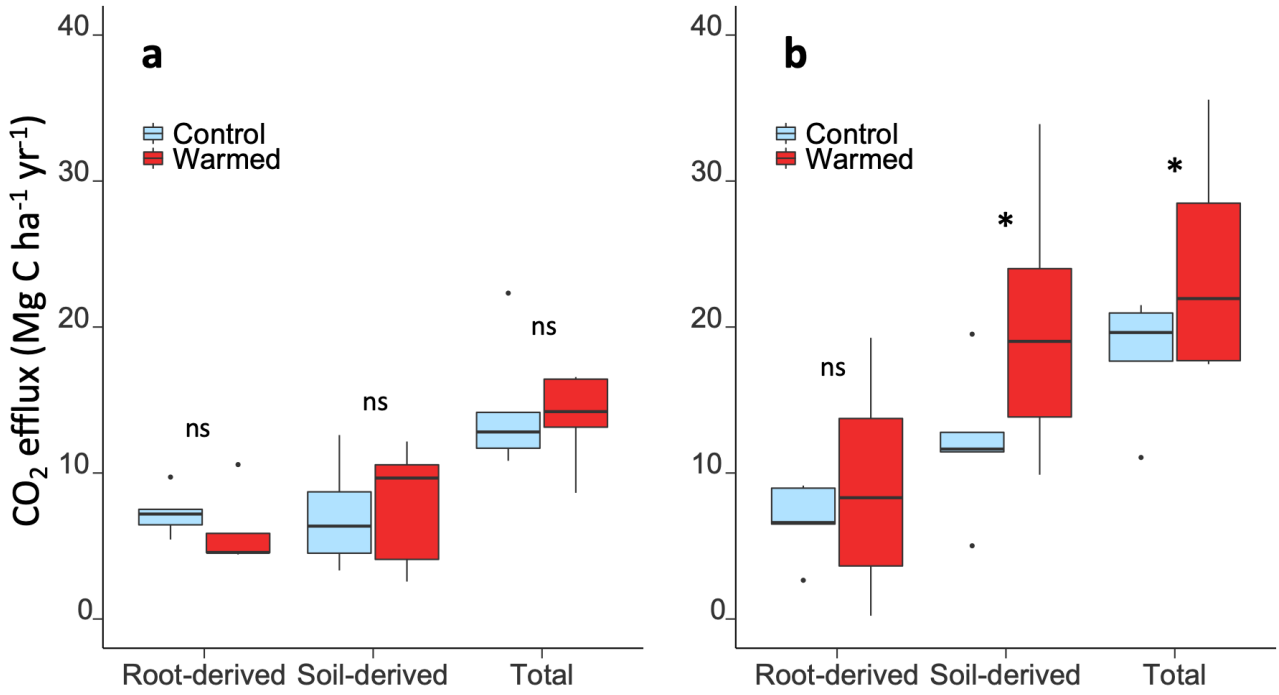

Figure 3 | The annual soil CO_2 efflux (total), partitioned into soil-derived and root-derived components. **a**. The pre-treatment period (Jan-May 2016; predominantly dry-season). **b.** The two years with warming. The figures show total CO_2 (Total) and the root-derived and soil-derived components calculated using CO_2 efflux from partition cores (equations 1-2). Differences between control and warmed plots are shown by asterisks where $p \leq 0.05$ or as non-significant (ns); determined using mixed effect models (Extended Data Tables 2-3). The error bars represent one standard error of the variation across the five plots over the study period.

METHODS

Site and experiment. The experiment is situated in approximately 1 ha area of seasonally moist lowland tropical forest on Barro Colorado Island, Panama[31]. Within the plot area the dominant tree species include *Anarcardium excelsum* and *Poulsenia armata*. The soils are Inceptisols (Fine, isohyperthermic, Dystric Eutrudepts) that are rich in clay (~54% profile-weighted clay concentration) and secondary metal oxides and are developed on the volcanic facies of the Bohio Formation, a basaltic conglomerate of Oligocene age[32]. Although these moderately weathered Inceptisols are less infertile than soils under large areas of lowland tropical forests, Inceptisols still account for 14% of total land area in the tropics (Ultisols and Oxisols account for 20% and 23%, respectively)[33], and soil respiration in the control plots in our experiment is comparable to that in lowland tropical forests in general, including those on Ultisols and Oxisols (see main text, ref. 20).

The SWELTR experiment consists of 10 circular plots (five paired plots 'warm' and 'control'). Each plot measures 5 m diameter, with approximately 10 m between each plot-pair and a minimum of 20 m between different plot-pairs. The warmed plots contain two heating structures, each consisting of eight 1-m long stainless-steel rods, connected by approximately 50 cm of flexible stainless-steel conduit. We used stainless-steel T-junctions at the top of each rod (adjoining the flexible conduit) and conical caps at the bottom of each rod. The final structure was 1.2 m tall. Inside each of the structures, we threaded 25 m of heating cable (SLMCAB10120BF, Briskheat, Columbus, USA) and filled the remaining space in the rods and conduit with quartz sand, selected for its high thermal conductivity. The complete structure was welded to seal the heating cable and sand inside. Two of these structures were buried around a 3.5 diameter circumference, with the top of the flexible conduit 5 cm belowground. Thus, each plot contained 50 m of heating cable inserted to 1.2 m depth, encircling a 3.5 m diameter area; with an effective heated plot area of 5 m diameter. The experiment heats

342 approximately 120 m^3 soil in total (5 plots x 5 m diameter by 1.2 m depth). The plot design and heating
343 methodology follows that of Hanson et al. (ref. 34) and Hicks Pries et al. (ref. 5).
344
345 ***Temperature control.*** Each warming and control plot was connected to a thermostat system, which
346 maintained soil temperature in the warmed plots at 4ºC above ambient temperature. The thermostat
347 system consisted of three integrated temperature and moisture sensors per plot (CS655 Reflectometer,
348 Campbell Scientific) inserted to 0-20, 50-70 and 100-120 cm depth at the mid-radius point in each
349 plot, which were connected to a control unit (one control unit for each plot pair; five in total). The
350 control units consisted of waterproof (IP68) enclosures containing a solid-state power controller
351 (DA10-24C0-0000, Watlow), relay (12V single channel), datalogger (CR1000, Campbell Scientific)
352 and 12V and 120V power supply. Temperature in each warmed plot was therefore maintained at 4ºC
353 above the temperature in each corresponding paired control plot, based on the average temperature
354 from 0-120 cm depth at the mid-radius point in each plot. The average temperature differential over
355 two years was 3.97ºC, which was the average of 2.7ºC at 0-20 cm depth, 4.0ºC at 50-70 cm depth and
356 5.2ºC at 100-120 cm depth.
357 This experimental design has been shown to warm the soil approximately uniformly across the
358 soil volume, with minor anomalies of warmer soil very close to the heating rods (< 10 cm) and slightly
359 cooler surface soils due to heat-transfer to the air[5, 34]. Therefore, surface soils were slightly cooler
360 compared to subsoils, although the response of surface rather than subsoils will likely dominate the
361 warming response across the soil profile because they contain greater organic matter (two thirds of the
362 C stock occurs in the upper 50 cm of the soil profile; Extended data Table 1). The heating structures
363 were installed during May-July 2016, and plots were tested during June-October 2016. The testing
364 phase consisted of heating each plot by 4ºC for approximately 2-week periods. The experiment was
365 switched on in full on 1 November 2016.
366

367 ***Soil gas-exchange and partitioning.*** Soil CO_2 efflux was measured every two weeks at four
368 systematically distributed locations within each plot from 2016 until 2019 using an infra-red gas
369 analyser (IRGA Li-8100; LI-COR Biosciences, Nebraska, USA). The soil collars for soil CO_2 efflux
370 measurements were assigned to zones within each plot ('centre 1', centre 2', 'side 1' and 'side 2') and
371 were relocated randomly within each zone every three months, for long-term within-plot spatial
372 independence. Soil CO_2 efflux was also measured every two weeks for four root-partition cores per
373 plot (2 × root-exclusion and 2 × root-ingrowth) to determine soil- and root-derived components of the
374 CO_2 efflux. At the same time as soil CO_2 efflux measurements, we measured soil temperature (using
375 a HI98509 thermometer probe; Hanna Instruments, USA) and soil moisture (using a Thetaprobe;
376 Delta-T, Cambridge, UK) at 0-20 cm soil depth for a random location within 1 m radius of each soil
377 collar, or within the root-partition cores.

378 Root-exclusion cores were made from PVC tubing (30 cm height, 10 cm diameter) with a 1
379 µm nylon mesh base for drainage. Root-ingrowth cores (i.e. disturbance controls) had additional
380 windows (~340 cm^2) covered with 2 mm mesh around the sides[35]. In each plot, two root-exclusion
381 cores and two root-ingrowth cores were buried within each plot, approximately 30 cm from the heating
382 cable (where the soil profile is warmed on average by 4^oC).

383 The percentage contributions of fine roots and rhizo-microorganisms (root-derived) and free-
384 living heterotrophic microorganisms (soil-derived) to the total soil CO_2 efflux were calculated as
385 follows:

386 Equation 1: Soil-derived (%) = (root-exclusion core CO_2 efflux / root-ingrowth core CO_2 efflux) x
387 100

388 Equation 2: Root-derived (%) = 100 - Soil-derived (%)

389

390 Total soil CO_2 efflux measured for soil collars was multiplied by the results from equations 1–2 to
391 estimate the absolute contributions of root-derived (roots, rhizo-microbial and mycorrhizal) and soil-

392 derived (free-living microbial through the decomposition of litter and soil organic matter)
393 components[35]. The partition cores were buried to 0-25 cm depth, where 95% fine roots occur[36] –
394 therefore the soil component consists of soil-derived CO_2 from the entire soil profile (with very
395 marginal contribution from fine roots at > 20 cm depth).
396
397 ***Soil properties.*** Soil was sampled before the experimental treatments began (to 100 cm depth;
398 Extended Data Table 1) and then every three months following the beginning of the experiment (0-10
399 cm; average responses in Extended Data Fig. 4) within the plots at a point where the surface soil is
400 evenly heated (at approximately 30 cm distance from the heating structure), and analysed for soil
401 properties: total elements, available nutrients, microbial CNP and enzyme activities using standard
402 procedures (see SI methods). We calculated microbial carbon-use-efficiency (CUE) using microbial
403 CNP and enzyme activity data using a stoichiometric method[37]. Here we describe the responses
404 following two years of warming, by using the average change in soil properties over two years (average
405 of eight temporal measurements per plot, with n = 5 per plot). Full details on these analytical methods
406 are provided in SI.
407
408 ***Statistical analyses.*** Treatment (warming) effects on time-averaged total and partitioned CO_2
409 emissions and other soil properties (nutrients, microbial properties), were tested using ANOVA.
410 Treatment effects on soil CO_2 emissions were further tested using mixed effects models with CO_2
411 emission as the response variable and warming-treatment, soil moisture, season, warming × soil
412 moisture and season × soil moisture as fixed effects and plot number as random effect[38]. We tested
413 both with and without a repeated measures effect because within-plot soil CO_2 efflux measurements
414 were partially spatially independent (within-plot locations of soil collars were changed every three
415 months, see soil gas exchange measurements above). Treatment effects on partitioned root- and soil-
416 derived CO_2 efflux components were tested using repeated measures mixed models with time and plot

number as random effects. Treatment effects on soil-surface moisture (0-20 cm depth) were tested using mixed effects models with soil moisture as the response variable and warming-treatment, season, warming × season as fixed effects and plot number as random effect. For whole-profile soil moisture we included time as an additional random effect (soil-surface moisture and temperature measurements were fully independent, whereas whole-profile measurements were repeated measures of fixed points). Further details on these approaches are provided in SI. All statistical analyses were performed in R (version 3.5.2).

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